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Effects of lauric and myristic acids on ruminal fermentation, production, and milk fatty acid composition in lactating dairy cows

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ABSTRACT

The objectives of this experiment were to investigate the effects of lauric (LA) and myristic (MA) acids on ruminal fermentation, production, and milk fatty acid (FA) profile in lactating dairy cows and to identify the FA responsible for the methanogen-suppressing effect of coconut oil. The experiment was conducted as a replicated 3 × 3 Latin square. Six ruminally cannulated cows (95 ± 26.4 DIM) were subjected to the following treatments: 240 g/cow per day each of stearic acid (SA, control), LA, or MA. Experimental periods were 28 d and cows were refaunated between periods. Lauric acid reduced protozoal counts in the rumen by 96%, as well as acetate, total VFA, and microbial N outflow from the rumen, compared with SA and MA. Ruminal methane production was not affected by treatment. Dry matter intake was reduced 35% by LA compared with SA and MA, which resulted in decreased milk yield. Milk fat content also was depressed by LA compared with SA and MA. Treatment had no effect on milk protein content. All treatments increased milk concentration of the respective treatment FA. Concentration of C12:0 was more than doubled by LA, and C14:0 was increased (45%) by MA compared with SA. Concentration of milk FA < C16 was 20% lower for LA than MA. Concentrations of *trans* 18:1 FA (except *trans* 12) and CLA isomers were increased by LA compared with SA and MA. Overall, the concentrations of saturated FA in milk fat were reduced, and that of > C16 FA and MUFA were increased, by LA compared with the other treatments. In this study, LA had profound effects on ruminal fermentation, mediated through inhibited microbial populations, and decreased DMI, milk yield, and milk fat content. Despite the significant decrease in protozoal counts, however, LA had no effect on ruminal methane production. Thus, the antimethanogenic effect of coconut oil, observed in related studies, is likely due to total FA application level, the additive effect of LA and MA, or a combination of both. Both LA and MA modified milk FA profile significantly.

Key words: dairy cow, lauric acid, myristic acid, milk fatty acid

INTRODUCTION

On a global scale, greenhouse gas (**GHG**) emissions from livestock amount to approximately 18% of all anthropogenic GHG emissions according to a report by the Food and Agriculture Organization of the United Nations (FAOUN, 2006). In some industrialized countries, however, enteric methane production by ruminants contributes only a small proportion of total GHG emissions. In the United States, for example, livestock contribution to GHG emissions is a mere 2.2%, based on US Environmental Protection Agency data (US EPA, 2007). These discrepancies can be easily explained by differences in the relative contributions of the energy, transportation, and livestock sectors in developed vs. developing countries (Hristov, 2008) and also by methodology inconsistencies (Pitesky et al., 2009). Irrespective of these disagreements, however, animal scientists are increasingly investigating methods for reducing enteric methane emissions from ruminant animals (Boadi et al., 2004; Bodas et al., 2008). Apart from being a pollutant, methane also represents a significant energy loss to the animal (Moe and Tyrrell, 1979).

Fatty acids (**FA**) have been investigated and may be an attractive tool to control rumen methanogenesis. In general, long-chain unsaturated and medium-chain saturated FA (**MCSFA**) were effective in inhibiting methanogens or decreasing methane production in vitro or in vivo (Henderson, 1973; Odongo et al., 2007; Johnson et al., 2009), but in some cases, the effects were biologically or statistically insignificant (Dohme et al., 2001; Beauchemin et al., 2009). Medium-chain saturated FA have strong antiprotozoal properties (Hristov et al., 2004a,b, 2009), and it has been suggested that the effects of defaunation and direct inhibition of archaea by MCSFA on methane suppression is additive (Dohme et al., 1999), although this association is not always clear (Karnati et al., 2009). Lauric (C12:0; **LA**) and myris-

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tic acids (C14:0; **MA**) compose approximately 63% of coconut oil, with about 10% palmitic and 7 to 8% oleic acids (CRC, 1988), and coconut oil has been effective in mitigating enteric methane production in ruminants. In a recent experiment with dairy cows, we found no effect on methane production with LA, but a significant reduction with coconut oil (Hristov et al., 2009). In sheep, 3.5 or 7.0% dietary inclusion of coconut oil reduced methane production by 27 and 73%, respectively (Machmüller and Kreuzer, 1999). Thus, supplementation of cattle diets with coconut oil may be a practically feasible method of mitigating enteric methane emission from ruminants and the environmental impact of livestock operations. It is important, however, to identify the methane-suppressing properties of the 2 major FA in coconut oil. Machmüller (2006) reported dramatic decreases (up to 50%) in methane production with both LA and MA at dietary inclusion levels below 3%. Odongo et al. (2007) reported a 36% reduction in methane production in dairy cows fed a diet supplemented with 5% MA. Other reports, however, found no effect of MA on ruminal methanogenesis (Dohme et al., 2001; Soliva et al., 2003). Then, there are some indications that the effect of LA and MA on methane production is additive (Soliva et al., 2004; Machmüller, 2006). Thus, it remains uncertain which FA is responsible for the antimethanogenic effect of coconut oil, or if the effect of LA and MA is additive.

Therefore, the objective of this study was to investigate the effect of LA and MA individually, on ruminal fermentation, methanogenesis, and production in lactating dairy cows. Our hypothesis, based on previous observations (Hristov et al., 2004a,b), was that LA is the FA primarily responsible for the methanogenesis inhibition observed with coconut oil (Hristov et al., 2009).

MATERIALS AND METHODS

Animals involved in this study were cared for according to the guidelines of The Pennsylvania State University Animal Care and Use Committee. The committee reviewed and approved the experiment and all procedures carried out in the study.

Animals and Experimental Design

Six multiparous Holstein cows (666 ± 14.4 kg of BW; 95 ± 26 DIM at the start of the trial) fitted with 10-cm ruminal cannulas (Bar Diamond, Parma, ID) were used. Cows were randomly assigned to experimental treatments in a replicated 3 × 3 Latin square design balanced for residual effects. Treatments were as follows: 240 g/cow per d each of stearic (**SA**, minimum 98% stearic acid; control), lauric (minimum 99% LA), or myristic (minimum 98% MA) acids. Lauric and myristic acids were from KIC Chemicals, Inc. (New Paltz, NY). Stearic acid was from Brenntag Nederland BV (Dordrecht, the Netherlands). The application level of 240 g/cow and application method (intraruminal) were chosen based on previous studies (Hristov et al., 2004b, 2009; Faciola et al., 2005, 2008). Stearic acid was used as a control treatment as it has minimal effects on ruminal fermentation (Hristov et al., 2004a). Treatments were applied as a pulse dose once a day (immediately before morning feeding) throughout the 21-d experimental periods, directly into the rumen via the cannula by mixing with approximately 5 kg of whole ruminal contents. The basal diet was fed as a TMR (Table 1). As formulated and at 27 kg/d of DMI, the diet exceeded the NE_L requirements of a Holstein cow yielding 45 kg of milk/d containing 3.40% milk fat and 3.00% milk true protein, but was deficient in RDP (−66 g/d) and MP (−269 g/d) according to NRC (2001). Cows were offered the daily ration as equal meals at 0800 and 2000 h. Diets were fed ad libitum in amounts resulting in 5% refusals. Each experimental period comprised a 21-d treatment adaptation and 7 d for sampling. On the last day of periods 1 and 2, all cows were transfaunated with approximately 20 kg/cow of whole ruminal contents from donor cows fed the same basal diet (not supplemented with FA). Following a 7-d refaunation period, the cows were assigned to new treatments. On d 7 of the refaunation period, a whole-ruminal-contents sample was collected before the a.m. feeding from the bottom of the rumen. The contents were squeezed through 2 layers of cheesecloth, and protozoa were counted as described below. Cows were housed in a tiestall barn during the entire trial and were exercised for about 3 h/d during regular milking (twice daily). Cows had continuous access to fresh water. One square (3 cows) received and the other (3 cows) did not receive recombinant bST (**rbST**) during the entire trial.

Sampling and Measurements

Samples of forage, TMR, and refusals were collected daily, and concentrate feeds were sampled weekly. Feed samples were composited and analyzed for DM (65°C in a forced-air oven, dried to a constant weight) ash/OM (AOAC, 2000), N (Kjeldahl; AOAC, 1980), NDF (Van Soest et al., 1991), and starch (starch assay kit, Megazyme International Ireland Ltd., Wicklow, Ireland; McCleary et al., 1994). A heat-stable α-amylase was used in the NDF analysis. Sodium sulfite was not used in the

analysis and NDF was expressed inclusive of residual ash. Composite TMR samples also were analyzed for acid-insoluble ash (Van Keulen and Young, 1977).

Whole ruminal contents samples were collected at 2, 4, 6, 8, 10, 14, 18, and 24 h following the morning feeding on d 23 of each experimental period. Ruminal samples were collected from 4 locations in the rumen and the reticulum (approximately 250 g each) and composited; aliquots were filtered through cheesecloth. Subsamples of the cheesecloth filtrates were immediately analyzed for pH and processed for analyses of ammonia (Chaney and Marbach, 1962), total free AA (ninhydrin procedure; Snell and Snell, 1954), VFA (Yang and Varga, 1989), and polysaccharide-degrading activities (carboxymethylcellulase, amylase, and xylanase; Hristov et al., 1998). Samples for protozoal enumeration were preserved (Hristov et al., 2001) and counted according to standard procedures (Dehority, 1993). Generic distribution of protozoa was determined from the total number of cells of each genera counted in 100 fields using the Sedgewick-Rafter chamber (Hausser Scientific, Horsham, PA).

Methane, nitrous oxide, and carbon dioxide production in the rumen were measured using the sulfur hexafluoride (SF_6) tracer technique (Johnson et al., 1994). The SF_6 permeation tubes were prepared by K. Johnson (Washington State University, Pullman) and placed in the reticulum of the cows on d 1 of the experiment. The tubes remained there for the duration of the entire study. Gas samples for methane analysis were collected directly from the rumen through modified rumen cannula lids as described elsewhere (Hristov et al., 2009) at 2, 3, 4, 5, and 6 h after the a.m. feeding on d 22 of each experimental period. Gas samples were analyzed for methane, nitrous oxide, and carbon dioxide, as well as SF_6 on a photoacoustic gas analyzer (INNOVA model 1412, AirTech Instruments, Ballerup, Denmark). Methane, carbon dioxide, and nitrous oxide production was calculated as the release rate of SF_6 times the ratio of the concentration of methane, carbon dioxide, or nitrous oxide to SF_6 in the ruminal headspace (Johnson et al., 1994).

Samples of whole ruminal contents (approx. 70 g) were stored frozen at −20°C for further DNA analysis. The samples were thawed and then mixed with an equal volume of phosphate buffer. The samples were homogenized (3 × 20 s) with a handheld Braun homogenizer (Bronwill Scientific Co., Rochester, NY). The liquid was separated from the remaining solids with a Bodum filter plunger (Bodum USA Inc., New York, NY). Five-milliliter samples of liquid were centrifuged 10 min at 10,000 × *g*. The supernatant was discarded and the remaining bacterial pellet was resuspended in 1.4 mL of ASL buffer (Qiagen Sciences, La Jolla, CA).

Table 1. Ingredient and chemical composition of the basal diet fed in the trial

Ingredient[1]	% of DM
Corn silage[2]	47.6
Grass hay[3]	10.2
Corn grain, ground	6.9
Canola meal, mechanically extracted	8.9
Soybean seeds, whole, heated	10.2
Cookie by-product[4]	5.9
Cottonseed hulls	2.2
Sugar blend[5]	4.2
Mineral/vitamin premix[6]	3.5
Optigen[7]	0.4
Composition,[8] % of DM or as indicated	
CP	14.4
Soluble protein, % of CP	31.7
RDP,[9] % of CP	65.8
NDF	34.8
ADF	21.6
NE_L, Mcal/kg	1.67
Starch	26.4
NFC	41.4
Ca	0.69
P	0.33

[1]In addition to the basal diet, cows received 240 g/d of fatty acids, which would add approximately 1.8 Mcal/d of digestible energy (based on the energy content of vegetable oil; NRC, 2001).

[2]Corn silage was on average 37.1% DM and (as % of DM): 34.7% NDF, 7.7% CP, and 42.1% starch.

[3]Grass hay contained (as % of DM): 75.2% NDF and 5.8% CP.

[4]Cookie by-product (Bakery Feeds, Honey Brook, PA) contained (as % of DM): 9% CP, 8% ether extract, and 5% crude fiber.

[5]Sugar blend (Westway Feed Products, Tomball, TX) contained (as % of DM): 3.9% CP and 66% total sugar.

[6]The premix contained (%, as-is basis): trace mineral mix, 0.88; MgO (54% Mg), 8.3; NaCl, 6.4; vitamin ADE premix, 1.73; limestone, 35.8; selenium premix, 1.09; and dry corn distiller grains with solubles, 45.8. Composition: Ca, 14.1%; P, 0.35%; Mg, 4.58%, K, 0.41%; S, 0.31%; Mn, 1,071 mg/kg; Cu, 358 mg/kg; Zn, 1,085 mg/kg; Fe, 181 mg/kg; Se, 6.67 mg/kg; Co, 5.4 mg/kg; I, 13.4 mg/kg; vitamin A, 262,101 IU/kg; vitamin D, 65,421 IU/kg; and vitamin E, 1,971 IU/kg.

[7]Optigen is a nonprotein nitrogen source (243.2% CP, DM basis) from Alltech, Inc. (Nicholasville, Kentucky).

[8]As analyzed by Cumberland Valley Analytical Services (Maugansville, MD).

[9]Estimated based on NRC (2001).

The DNA was then extracted using a Qiagen DNA kit (Qiagen Sciences) according to the manufacturer's protocol and eluted in 50 μL of Tris- EDTA buffer (0.1 *M* Tris, 1 m*M* EDTA; pH 7.5). The DNA concentration was measured using a PicoGreen dsDNA quantitation kit (molecular probes, Invitrogen, Eugene, OR) in a multidetection microplate reader (model SIAFRM, Bio-Tek Instruments Inc., Winooski, VT) with calf thymus DNA (Sigma-Aldrich, St. Louis, MO) as the standard. Concentrations were adjusted to 10 ng/μL and used for further experiments.

To evaluate diversity differences in the archaeal populations, DNA fingerprinting profiles of the archaeal

communities were generated using denaturing gradient gel electrophoresis (**DGGE**). The DGGE was performed with an approximately 500-bp fragment of the 16S rRNA gene (v3–v6 region) as described by Ohene-Adjei et al. (2007). The DNA from individual animals was used as the template. Briefly, PCR was conducted using Arch 344F (ACG GGG YGC AGC AGG CGC GA) and Arch 915R (GTG CTC CCC CGC CAA TTC CT) primers. The forward primer had an added 40-bp GC clamp (Muyzer and Smalla, 1998). The final volume (25 uL) of all PCR reactions contained 20 ng of template, 2.5 μL of 10× dilution buffer, 10 pmol of each primer, and 1 U of Taq polymerase (Takara Shuzo, Japan). All PCR amplifications were performed using a 96 well i-Cycler (Bio-Rad Laboratories, Hercules, CA). The amplification conditions involved denaturation at 95°C for 1 min, followed by 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min.

Quantitative PCR was used to evaluate the population size of total archaea as described by Ohene-Adjei et al. (2007). Quantitative PCR was performed on a 96-well ABI 7900HT (Applied Biosystems, Foster City, CA) using SYBR Green Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The Arch 896–915 F (AGG AAT TGG CGG GGG AGC AC) and the Arch 1406–1389 R (ACG GGC GGT GTG TGC AAG) primer sets were used for the quantitation of total archaea. A reaction mix (20 μL) contained 20 ng of genomic DNA and 10 pmol of each primer. The cycling conditions were 40 cycles of 95°C for 15 s, and 60°C for 60 s.

Fecal samples (400 g/sampling) were collected from the rectum or the ground (when fresh) during the first 2 d of each sampling period at 0900, 1500, and 2100 h (d 22), and at 0300, 0600, 1200, 1800, and 0000 h (d 23 and 24). Samples were dried at 65°C in a forced-air oven to constant weight, composited per animal and period, and ground through a 1-mm sieve. Samples were analyzed for ash (OM), N, NDF, starch, and acid-insoluble ash. Apparent total-tract digestibility was estimated using acid-insoluble ash as an intrinsic marker (Foley et al., 2006). At each sampling, an additional fecal sample (approximately 300 g) was collected, composited (per cow and treatment), and immediately frozen (−20°C) for analysis of gaseous emissions from manure.

Total urine collection was performed during the last 4 d of each experimental period. Urinary catheters (22 French, 75 mL, C. R. Bard Inc., Covington, GA) were positioned in the cows 24 h before the initiation of the urine collection. Urine samples were acidified during collection to a pH <3.0 by addition of 2 *M* sulfuric acid. Aliquots were diluted 1:10 with distilled water, stored frozen at −20°C, and later analyzed for N, allantoin (Chen, 1989), and uric acid (uric acid kit 1051, Stanbio Laboratory, San Antonio, TX). Urinary excretion of purine derivatives, allantoin and uric acid, was used to estimate duodenal microbial N flow. At the beginning of each urine collection period, a nonacidified sample of urine (approximately 2 L) was collected from each cow, frozen immediately (−20°C), and subsequently used for the analysis of gaseous emissions from manure.

Ammonia, methane, and nitrous oxide emitting potential of manure resulting from the respective diet changes were measured in a steady-state gas emission system (Wheeler et al., 2007). Emission measurements in this experiment were conducted at 25°C with 2-L/min sweep airflow. Feces and urine (see above paragraphs) were thawed and mixed 1.7:1 (252 g of feces and 148 g of urine per jar) and incubated for 165 h. The feces:urine ratio was chosen based on experiments from the senior author's laboratory, in which fecal and urinary N output was measured in lactating dairy cows. Ammonia, methane, and nitrous oxide concentrations in the gas leaving the chambers were measured with an INNOVA gas analyzer. Gas measurements were taken every 30 min. Nitrous oxide concentration in manure gas was negligible in this experiment (around 0.6 mg/m^3) and nitrous oxide emissions will not be further discussed.

Milk yield data were collected daily and milk samples (a.m. and p.m. milkings) for composition analyses (Dairy One, Ithaca, NY) were collected on 3 separate days during the last 2 wk of each period. Milk samples for fatty acid composition were collected and stored at −20°C until analyzed according to Hristov et al. (2010).

On d 27 of each experimental period, blood samples were collected from the tail vein/artery immediately before and 6 h after morning feeding. Plasma was obtained by centrifugation at 1,500 × *g* for 40 min, frozen at −40°C, and later analyzed for urea N (kit 0580, Stanbio Laboratory) and glucose (kit 1075, Stanbio Laboratory) concentrations.

Body weight of the cows was recorded throughout the experiment. Urinary purine derivatives excretion was used to estimate duodenal microbial N flow (for equations, see Hristov et al., 2009). A ratio of purine N to total N in rumen microorganisms of 0.134 was assumed based on the data of Valadares et al. (1999).

Statistical Analysis

Data were analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC). Intake, digestibility, ruminal enzymatic activities and protozoal counts, urinary excretion measurements, milk yield and composition, and end-point (165 h) cumulative ammonia and methane emission from manure data were analyzed by

ANOVA Latin square. Milk composition data collected during each experimental period were averaged per cow, and the average values were used in the statistical analysis and to calculate FCM, milk NE_L, and milk fat and protein yields. Data were analyzed according to the following model:

$$Y_{ijkl} = \mu + G_i + C(G)_{ij} + P_k + \tau_l + e_{ijkl}, \quad [1]$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, G_i is the group, $C(G)_{ij}$ is the cow within group, P_k is the kth period, τ_l is the lth treatment, with the error term e_{ijkl} assumed to be normally distributed with mean = 0 and constant variance. Group and cow within group were random effects, whereas all other factors were fixed.

Ruminal pH, ammonia and VFA concentrations, and methane, carbon dioxide, and nitrous oxide production data were analyzed as Latin square repeated measures assuming an autoregressive(1) covariance structure fitted on the basis of Akaike information and Schwarz Bayesian model-fit criteria according to the following model:

$$Y_{ijklm} = \mu + G_i + C(G)_{ij} + P_k + \tau_l + D_m + \tau D_{lm} + e_{ijklm}, \quad [2]$$

where Y_{ijklm} is the dependent variable, μ is the overall mean, G_i is the group, $C(G)_{ij}$ is the cow within group, P_k is the kth period, τ_l is the lth treatment, D_m is the time effect, τD_{lm} is the treatment × time interaction, with the error term e_{ijklm} assumed to be normally distributed with mean = 0 and constant variance. Group and cow within group were random effects, whereas all else were fixed.

Cumulative ammonia and methane emissions from manure data were fitted (PROC NLIN, SAS) to a single rectangular 2-parameter hyperbola model of the type f = a × x ÷ (b + x), in which f was cumulative ammonia or methane emission (mg), a represented the theoretical maximum of ammonia or methane emissions , x was incubation time (h), and b was the regression constant. The average proportion of the variance explained by the models (regression sum of squares ÷ uncorrected total sum of squares) was 0.98 ± 0.001 and 0.96 ± 0.007 for ammonia and methane respectively. Estimated maximum emission and overall emission lines were compared between treatments using the dummy variable regression technique (PROC NLMIXED, SAS; Bates and Watts, 1988).

Statistical differences were declared at $P \leq 0.05$. Differences between treatments at $P \leq 0.10$ were considered as a trend toward significance.

RESULTS

Rumen pH was higher ($P = 0.049$) for LA compared with SA and MA (Table 2); no treatment × time of sampling interaction was observed. Ammonia concentration in ruminal fluid was decreased ($P = 0.044$) by LA compared with MA and tended to be decreased ($P = 0.084$) compared with SA. Rumen ammonia concentration was lower for LA after the p.m. feeding, but similar to the other treatments during the rest of the sampling period (Figure 1). There was no effect of treatment on concentration of total free AA. Lauric acid had a dramatic effect on ruminal fermentation by decreasing ($P = 0.007$ and < 0.001) concentrations of total VFA and acetate compared with SA and MA. Myristic acid had no effect on VFA. Total protozoal counts were reduced (96%; $P < 0.001$) by LA compared with SA and MA. The same effect was observed for *Entodinium* spp. Other protozoal species were not detected in the LA-treated cows and were low in the other treatments. Fibrolytic (carboxymethylcellulase and xylanase) activities were not reduced by LA compared with the other treatments, and amylase activity of ruminal contents was increased ($P = 0.043$) by LA compared with SA.

Concentration of nitrous oxide in the rumen gas phase was very low compared with that of methane and carbon dioxide, but was reduced ($P = 0.020$), particularly 2 h after the a.m. feeding (Figure 2), by LA compared with SA and MA. Production rate of nitrous oxide was about 0.2% of that of methane and was reduced ($P = 0.002$) by LA compared with SA and MA 2 h after feeding (treatment × time interaction, $P = 0.065$). Lauric acid also reduced ($P = 0.039$) ruminal concentration of carbon dioxide compared with SA and tended to reduce it ($P = 0.098$) compared with MA. Production rate of carbon dioxide was not affected by treatment. Concentration and production rate of methane were also not affected by treatment. It appears methane concentration tended to be lower for LA throughout the sampling period (Figure 3), but the differences did not reach statistical significance because of the large variability in the data.

Quantitative PCR was used to determine the effect of the treatments on the 16S rRNA copy number of methanogenic archaea. Statistical analysis of the data indicated that there were no significant differences in the estimated copy numbers of methanogenic archaea between treatments. Additionally, DGGE analysis did not show any variance in the number or size distribution of DNA bands between animals or diets, indicating that the population structure of the methanogenic archaea in the rumen did not change (data not shown).

Table 2. Effect of medium-chain saturated fatty acids[1] on rumen fermentation and BUN in dairy cows (least squares means; n = 160, rumen pH, ammonia, and VFA data; n = 90, rumen methane , nitrous oxide, and carbon dioxide; and n = 18, all other variables)

Item	SA	LA	MA	SEM	*P*-value
Rumen					
pH	6.17[b]	6.36[a]	6.13[b]	0.056	0.049[2]
Ammonia, m*M*	4.03[ab]	3.10[b]	4.41[a]	0.324	0.044[3]
Total free AA, m*M*	4.86	4.72	5.26	0.395	0.57
Total VFA,[4] m*M*	114.0[a]	104.6[b]	114.9[a]	2.20	0.007
Acetate	67.9[a]	58.5[b]	67.4[a]	1.22	<0.001
Propionate	27.9	27.1	28.7	1.40	0.64
Isobutyrate	1.22[a]	1.11[b]	1.21[a]	0.023	0.001
Butyrate	12.6	12.5	13.5	0.44	0.29
Isovalerate	1.87[b]	2.22[a]	1.92[b]	0.081	0.022
Valerate	2.43[b]	3.16[a]	2.52[b]	0.173	0.002[5]
Acetate:propionate	2.53	2.23	2.47	0.170	0.06[6]
Total protozoa, $\times 10^4$/mL	88.0[a]	3.8[b]	102.7[a]	18.72	<0.001[7]
Entodinium spp.	83.9[a]	3.8[b]	97.0[a]	18.16	<0.001
Isotricha spp.	0.29	ND[8]	0.32	0.102	0.11
Dasytricha spp.	1.15[a]	ND[b]	1.12[a]	0.363	0.037
Epidinium spp.	3.20	ND	4.80	0.745	0.067
Ophryoscolex spp.	ND	ND	0.03	0.015	0.41
Diplodinium spp.	1.63[a]	ND[b]	2.67[a]	0.578	0.019
PSD activities[9]					
CMCase	56	108	80	19.1	0.30
Xylanase	167	164	185	11.9	0.52
Amylase	86[b]	133[a]	102[ab]	9.9	0.043[10]
Rumen gases[11]					
Nitrous oxide, mg/m^3	91[a]	70[b]	93[a]	15.9	0.02
Nitrous oxide production rate, mg/h	31	26	30	1.9	0.17[12]
Methane $\times 10^3$, mg/m^3	35.3	30.9	38.5	4.29	0.13
Methane production rate, g/h	12.5	12.1	13.0	1.38	0.21
Carbon dioxide $\times 10^3$, mg/m^3	172[ab]	150[b]	183[a]	18.9	0.039[13]
Carbon dioxide production rate, g/h	61.9	61.7	61.9	6.78	0.98
Methanogen 16S rRNA[14]	10.0	11.3	10.8	1.43	0.84
Urinary PD,[15] mmol/d					
Allantoin	473[a]	328[b]	440[a]	25.7	0.002
Uric acid	48.5[a]	28.5[b]	43.9[a]	4.75	0.008
Total PD	521[a]	356[b]	484[a]	23.8	0.002
MN,[16] g/d	348[a]	226[b]	321[a]	16.5	0.002
BUN,[17] mg/100 mL	9.5	10.1	8.6	0.53	0.20
0 h	9.5	8.4	8.6	0.60	0.54
6 h	9.6[b]	11.9[a]	8.6[b]	0.76	0.006
Plasma glucose,[18] mg/100 mL	60.8	60.8	59.4	2.66	0.73

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).
[1]SA = stearic acid; LA = lauric acid; MA = myristic acid.
[2]Treatment × time interaction, $P = 0.69$.
[3]Treatment × time interaction, $P = 0.018$; LA vs. SA, $P = 0.084$.
[4]Treatment × time interactions for VFA, except valerate, $P = 0.09$ to 0.69.
[5]Treatment × time interaction, $P = 0.006$.
[6]LA vs. SA, $P = 0.024$.
[7]Actual protozoal counts were $\log_{10}$-transformed for the statistical analysis.
[8]Low counts = below 0.02×10^4/mL.
[9]PSD = polysaccharide-degrading activities. Expressed as nanomoles of reducing sugars as glucose released/mL of ruminal fluid per minute; CMCase = carboxymethylcellulase.
[10]LA vs. MA, $P = 0.082$.
[11]Average concentration of gases in rumen gas phase across sampling points (see Materials and Methods); Treatment × time interactions, $P = 0.10$ to 0.61.
[12]Treatment × time interactions, $P = 0.065$; at 2 h after feeding, LA vs. MA and SA, $P = 0.002$.
[13]LA vs. SA, $P = 0.098$.
[14]Methanogen archaea 16S rRNA copy, number $\times 10^4$/ng of total DNA.
[15]Excretion of urinary purine derivatives (PD).
[16]Estimated microbial N outflow from the rumen (based on urinary allantoin excretion).
[17]Averaged blood plasma urea N, or 0 h (before a.m. feeding) and 6 h after the a.m. feeding.
[18]Averaged blood plasma glucose (0 h and 6 h samples did not differ among treatments; $P = 0.67$ and 0.85, respectively).

Urinary excretion of allantoin and uric acid, and the estimated outflow of microbial protein from the rumen, were reduced ($P = 0.002$) by LA compared with the other treatments. The average concentration of PUN was not different among treatments, but was greater ($P = 0.006$) for LA compared with SA and MA 6 h after feeding. Plasma glucose concentration was unaffected by treatment.

Intake of DM was drastically reduced ($P = 0.002$) by LA compared with SA and MA, and similar trends were observed for the other nutrients (Table 3). Total-tract apparent digestibility of dietary nutrients was not affected by treatment. Urinary N excretion did not differ among treatments, but fecal and total N excretions were reduced ($P = 0.008$ and $P = 0.01$, respectively) by LA compared with SA and MA (Table 4), which was partially due to reduced N intake but also to proportionally greater (trend of $P = 0.111$) N losses in urine with the former treatment.

Milk yield was decreased ($P = 0.017$) by LA compared with the other treatments due to decreased DMI as milk feed efficiency was greater ($P = 0.009$) for LA than SA and MA (Table 5). When expressed on an FCM or milk NE_L basis, however, LA had no effect on feed efficiency. Milk fat content and yield were decreased ($P = 0.021$ and 0.016, respectively) by LA, which is indicative of milk fat depression. Expressed on a DMI basis, milk fat yield was not statistically different among treatments (although numerically lower for LA). Milk true protein concentration tended to be reduced ($P = 0.09$) by LA compared with SA, and true protein and N yields were reduced ($P = 0.016$) by LA compared with the other treatments. Milk lactose concentration was not affected by treatment, but lactose yield was reduced ($P = 0.018$) by LA compared with SA and MA. Milk protein and lactose yields per unit of DMI were greater ($P = 0.022$ and 0.024, respectively) for LA compared with SA. Milk NE_L output also was lower ($P = 0.009$) for LA, which was due to the lower energy intake with this treatment, but milk NE_L efficiency was not different among treatments. Lauric acid tended to increase ($P = 0.054$) MUN compared with SA.

Because one group of cows in the Latin square received rbST and the other did not, the interaction group × treatment was examined for all variables. The interaction was statistically significant ($P = 0.032$) only for milk fat yield (and the resultant variable, milk fat yield per unit of DMI). Milk fat yield was statistically lower ($P = 0.041$) for LA versus SA cows for the no-rbST group, but not for the rbST group ($P = 0.13$). As ranking among treatments was similar for both groups, and milk fat yield for LA cows was similarly decreased (60 to 63%) compared with SA cows, this interaction was considered to be of no biological significance.

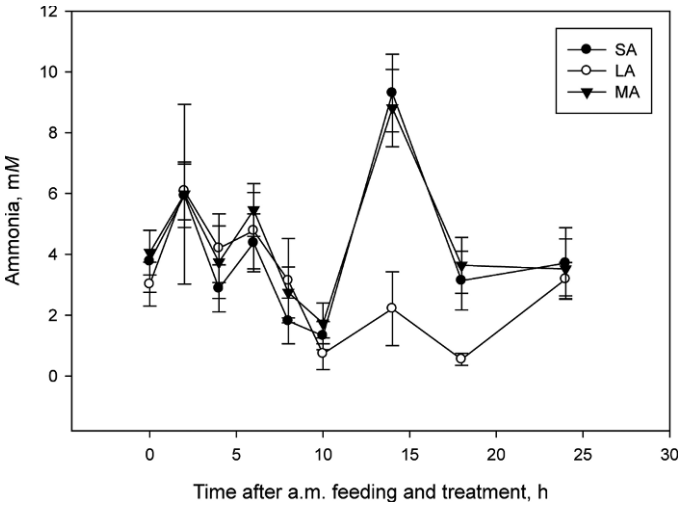

Figure 1. Effect of stearic (SA), lauric (LA), and myristic acids (MA) on ruminal ammonia concentration in dairy cows (means ± SE; n = 160). Overall treatment effect, $P < 0.044$; time of sampling effect, $P < 0.001$; treatment × time interaction, $P = 0.018$.

As expected, milk FA concentrations of treatment FA increased due to SA, LA, or MA administration. Milk fat content of 12:0 was more than doubled ($P < 0.001$) by LA and 14:0 was increased ($P < 0.001$) by MA compared with SA (Table 6). Treatment FA also influenced milk FA composition more broadly, and this was especially evident with the LA treatment. In general, concentration of the short-chain FA (4:0 to 10:0) was reduced ($P < 0.001$) by LA and in some cases by MA compared with SA. Myristoleic acid (14:1) also was increased with MA administration. Concentrations of

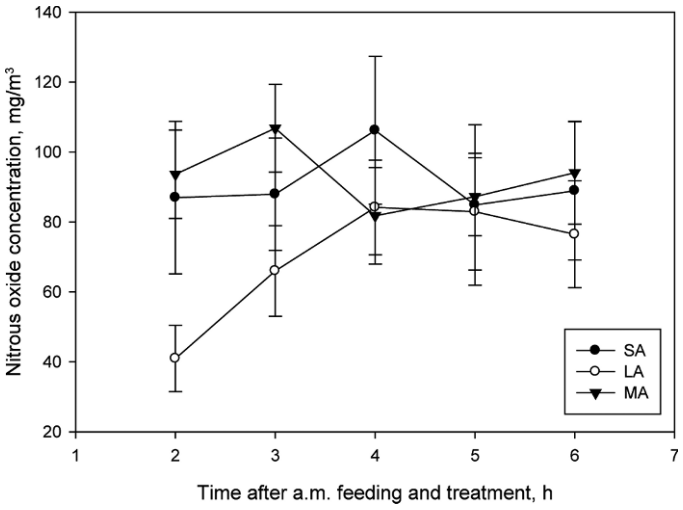

Figure 2. Effect of stearic (SA), lauric (LA), and myristic acids (MA) on nitrous oxide concentration in rumen gas of dairy cows (means ± SE; n = 90). Overall treatment effect, $P = 0.02$; time of sampling effect, $P = 0.29$; treatment × time interaction, $P = 0.10$.

Table 3. Effect of medium-chain saturated fatty acids[1] on intake and total-tract apparent digestibility of nutrients in dairy cows (least squares means; n = 18)

Item	SA	LA	MA	SEM	*P*-value
Intake, kg/d					
DM	26.9[a]	20.0[b]	25.7[a]	0.84	0.002
OM	25.3	18.7	24.1	0.79	0.005
N	0.627[a]	0.463[b]	0.592[a]	0.0186	0.001
NDF	9.4[a]	6.9[b]	9.0[a]	0.30	0.002
ADF	5.8[a]	4.3[b]	5.6[a]	0.19	0.002
Starch	7.1[a]	5.3[b]	6.8[a]	0.21	0.001
Apparent digestibility, %					
DM	65.4	67.7	67.3	2.28	0.75
OM	66.5	68.6	68.4	2.21	0.74
N					
NDF	43.9	42.1	45.6	3.77	0.81
ADF	31.8	32.1	33.9	4.00	0.91
Starch	98.1	98.6	97.8	0.30	0.33

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]SA = stearic acid; LA = lauric acid; MA = myristic acid.

major milk FA such as 16:0 and 18:0 were reduced ($P = 0.007$ and <0.001, respectively) by LA compared with the other treatments. Concentrations of *trans* 18:1 FA (except *trans* 12) and CLA isomers were increased ($P \leq 0.003$) by LA compared with SA and MA. Overall, the concentrations of saturated FA in milk fat were reduced ($P = 0.014$) and that of > C16 FA and MUFA were increased ($P = 0.004$) by LA compared with the other treatments.

The ammonia-emitting potential of manure was greatest ($P < 0.001$) for LA, lowest for SA, and intermediate for MA (Figure 4). The methane-emitting potential of manure was greatest ($P < 0.001$) for MA, lowest for LA, and intermediate for SA (Figure 5). The differences were small, particularly for methane, but highly significant due to the large number of measurements taken during the course of the incubation.

DISCUSSION

According to NRC (2001), and based on actual forage analyses, the basal diet was deficient in RDP and MP. Our analyses and experimental data (Huhtanen and Hristov, 2009; Lee and Hristov, 2010) and data from this experiment (cows reached their expected production level), however, suggest that NRC (2001) is likely overestimating protein requirements, particularly RDP requirements. Furthermore, inhibition of protozoa in the rumen is more likely to benefit the overall N efficiency of the cow only in protein deficient situations (Hristov and Jouany, 2005). Therefore, dietary protein level was kept intentionally low in this experiment.

The effect of LA on ruminal fermentation was profound. In agreement with our previous observations (Hristov et al., 2004b, 2009), protozoal counts were reduced by LA to about 4% of the control (SA) or MA counts. Except for *Entodinium* spp., no other genera were found in LA-treated cows. By contrast, the other major FA in coconut oil, MA, had no effect on protozoal counts, which is in agreement with our in vitro data (Hristov et al., 2004a) and data from Soliva et al. (2004) in the rumen simulation technique. Matsumoto et al. (1991), however, observed complete defaunation in 3 d in goats consuming about 5% (dietary DM basis) MA. At a much higher dose than in the present experiment (50 g/kg of dietary DM, which would correspond to about 1,350 g/cow in our study), MA significantly reduced protozoal counts in sheep (Machmüller et al., 2003). The effects of LA on ruminal pH, ammonia, and VFA are indicative of effective depression of microbial activities and, for the most part, are similar to our

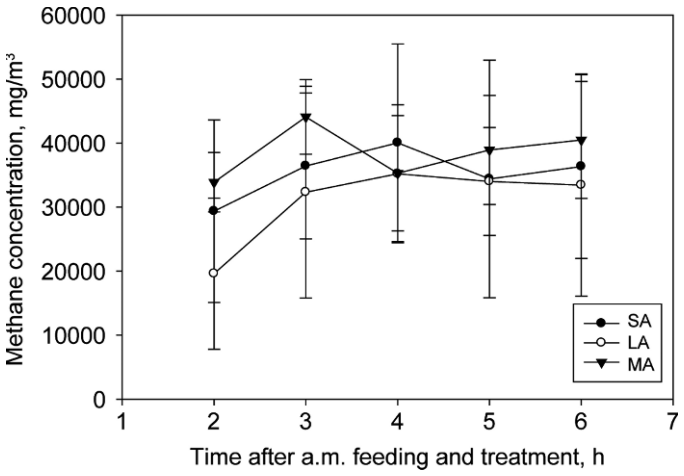

Figure 3. Effect of stearic (SA), lauric (LA), and myristic acids (MA) on methane concentration in rumen gas of dairy cows (means ± SE; n = 90). Overall treatment effect, $P = 0.13$; time of sampling effect, $P = 0.013$; treatment × time interaction, $P = 0.61$.

Table 4. Effect of medium-chain saturated fatty acids[1] on urinary and fecal N excretions in dairy cows (least squares means; n = 18)

Item	SA	LA	MA	SEM	*P*-value
Urinary N					
g/d	117	113	120	9.7	0.86
As % of N intake	19.0	24.8	20.4	2.49	0.22
As % of total excreted N	32.6	43.3	35.4	3.59	0.111[2]
Urea N, g/d	65.8	60.5	67.2	5.48	0.60
Fecal N					
g/d	241[a]	149[b]	221[a]	14.5	0.008
As % of N intake	39.0	32.1	37.7	2.41	0.21
Total N losses					
g/d	357[a]	262[b]	341[a]	14.1	0.01
As % of N intake	58.1	56.8	58.0	2.37	0.94

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]SA = stearic acid; LA = lauric acid; MA = myristic acid.

[2]LA vs. SA, $P = 0.048$.

previous observations (Hristov et al., 2009). One surprising effect of LA was the increased amylase activity of the ruminal contents (compared with SA). Given the apparently inhibited rumen microbial ecosystem with LA, there is no rational explanation for the increased amylase activity, except that amylolytic species may have been proportionally less affected by LA than other generalists and benefited from available substrate.

The decreased microbial production in the rumen with LA is in agreement with the numerically or statistically significant reductions in microbial flow we previously reported for LA and coconut oil (Hristov et al., 2004b, 2009). As in previous studies, the decrease in microbial protein flow with LA is mostly due to decreased DMI. For example, on an OM-intake basis, microbial N flow with LA was approximately 14% lower than that with

Table 5. Effect of medium-chain saturated fatty acids[1] on milk yield and composition in dairy cows (least squares means, n = 18)

Item	SA	LA	MA	SEM	*P*-value
Milk yield, kg/d	44.6[a]	35.8[b]	44.2[a]	1.70	0.017
Milk/DMI	1.63[b]	1.91[a]	1.81[b]	0.061	0.009
Milk fat, %	3.42[a]	2.59[b]	3.12[ab]	0.327	0.021[2]
Yield, kg/d	1.48[a]	0.92[b]	1.38[a]	0.173	0.012
Yield/DMI, g/kg	54.9	48.9	56.0	6.71	0.28
4% FCM, kg/d	39.6[a]	28.1[b]	38.3[a]	3.16	0.010
4% FCM/DMI	1.48	1.50	1.56	0.121	0.56
Milk true protein, %	2.98	2.90	2.89	0.032	0.09[3]
Yield, kg/d	1.30[a]	1.04[b]	1.27[a]	0.055	0.016
Yield/DMI, g/kg	48.4[b]	55.2[a]	51.8[ab]	1.90	0.022
N yield,[4] g/d	204[a]	162[b]	200[a]	8.7	0.016
As % of N intake	32.6	34.9	33.9	1.08	0.24
Milk lactose, %	4.84	4.78	4.78	0.135	0.18
Yield, kg/d	2.11[a]	1.71[b]	2.11[a]	0.119	0.018
Yield/DMI, g/kg	78.9[b]	91.6[a]	86.2[ab]	5.00	0.024
Milk NE_L,[5] Mcal/d	29.4[a]	21.1[b]	28.3[a]	2.31	0.009
Milk NE_L/DMI, Mcal/kg	1.09	1.13	1.15	0.090	0.58
NE_L intake,[6] Mcal/d	46.1[a]	34.4[b]	44.0[a]	1.40	0.002
Milk NE_L as % of NE_L intake	63.5	60.7	64.9	4.91	0.56
MUN, mg/100 mL	7.7	9.7	8.3	0.52	0.054[7]
BW, kg	672	665	661	45.2	0.36

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]SA = stearic acid; LA = lauric acid; MA = myristic acid.

[2]LA vs. MA, $P = 0.053$.

[3]LA vs. SA, $P = 0.061$; MA vs. SA, $P = 0.050$.

[4]Milk true protein yield ÷ 6.38.

[5]Milk NE_L (Mcal/d) = kg of milk × (0.0929 × % fat + 0.0563 × % true protein + 0.0395 × % lactose) (NRC, 2001).

[6]Based on NE_L concentration of the basal diet (Table 1), DMI (Table 3), and calculated NE_L intake with SA, LA, and MA (NRC, 2001, at actual DMI).

[7]LA vs. SA, $P = 0.021$; LA vs. MA, $P = 0.082$.

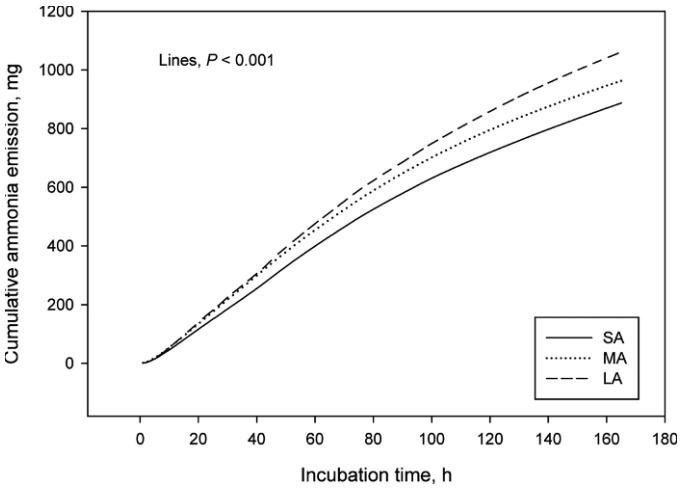

Figure 4. Effect of stearic (SA), lauric (LA), and myristic acids (MA) on cumulative ammonia emission from dairy manure. End-point (165 h) cumulative ammonia emission (n = 18; $P = 0.073$): 887, 1,061, and 962 mg (SA, LA, and MA, respectively). Comparison of regression lines (n = 3,366), $P < 0.001$.

SA (12.1 vs. 13.8 g/kg of OM, respectively). Numerical trends for depressed DMI with LA (or coconut oil) was also observed in our previous experiments (Hristov et al., 2004b, 2009) and was reported by others (Faciola et al., 2005, 2008; Hollmann and Beede, 2008), but the effect was more pronounced in this experiment. Similar to our previous studies (Hristov et al., 2004b, 2009), total-tract nutrient digestibility did not seem to be affected by LA (or MA). Others have not found a depression in digestibility of DM, OM, or fiber fractions with LA, MA, or coconut oil (Machmüller et al., 2003; Dohme et al., 2004; Jordan et al., 2006). Thus, it appears that palatability and reduced DMI are the major factors leading to decreased microbial protein synthesis with MCSFA or coconut oil, which perhaps counteracts the beneficial effect of inhibited protozoal population on microbial protein production in the rumen (Hristov and Jouany, 2005). The reduced DMI with LA was responsible for the reduced milk yield in this experiment. Cows treated with LA more efficiently utilized

Table 6. Effect of medium-chain saturated fatty acids[1] on milk fatty acid composition (g/100 g of total fatty acids) in dairy cows (least squares means; n = 18)

Fatty acid	SA	LA	MA	SEM	*P*-value
4:0	5.03[a]	3.64[b]	4.75[a]	0.321	<0.001
6:0	2.48[a]	1.49[c]	2.23[b]	0.208	<0.001
8:0	1.33[a]	0.75[c]	1.17[b]	0.124	<0.001
10:0	2.79[a]	1.64[b]	2.46[a]	0.264	<0.001
12:0	2.93[b]	6.27[a]	2.67[b]	0.263	<0.001
14:0	9.45[b]	7.66[c]	13.58[a]	0.349	<0.001
14:1	0.71[b]	0.86[a]	1.08[a]	0.135	0.007
15:0	0.69	0.65	0.68	0.040	0.73
16:0	22.6[a]	20.1[b]	22.3[a]	0.42	0.007
16:1	1.07[b]	1.60[a]	1.44[b]	0.188	<0.001
17:0	0.36	0.36	0.33	0.023	0.18
18:0	13.2[a]	9.7[c]	11.7[b]	1.11	<0.001
18:1 *trans*-6–8	0.56[b]	1.31[a]	0.57[b]	0.121	0.003
18:1 *trans*-9	0.39[b]	0.80[a]	0.40[b]	0.061	0.002
18:1 *trans*-10	0.74[b]	5.29[a]	1.20[b]	0.558	0.001
18:1 *trans*-11	1.67[b]	2.60[a]	1.69[b]	0.119	0.003
18:1 *trans*-12	0.68	0.75	0.67	0.034	0.27
18:1 *cis*-9	25.0	25.4	23.4	1.08	0.47
18:2 *cis*-9, *cis*-12	4.35	4.23	4.08	0.164	0.41
CLA[2] *cis*-9, *trans*-11	0.62[b]	1.13[a]	0.66[b]	0.139	<0.001
CLA *trans*-10, *cis*-12	ND[3b]	0.02[a]	0.004[b]	0.003	0.001
18:3	0.43[a]	0.38[b]	0.39[b]	0.009	0.038
20:0	0.15[a]	0.12[b]	0.14[ab]	0.008	0.022
Σ unidentified	2.71[b]	3.20[a]	2.63[b]	0.138	<0.001
Σ *trans*-18:1	4.04[b]	10.7[a]	4.52[b]	0.709	<0.001
Σ <C16	25.4[b]	23.0[b]	28.6[a]	1.01	0.011
Σ C16	23.6[a]	21.7[b]	23.5[a]	0.54	0.027
Σ >C16	50.9[b]	55.3[a]	47.9[b]	1.12	0.014
Σ saturated fatty acids	61.0[a]	52.4[b]	62.0[a]	2.01	0.003
Σ MUFA[4]	30.9[b]	38.6[a]	30.2[b]	1.65	0.004
Σ PUFA[4]	5.39[ab]	5.76[a]	5.14[b]	0.289	0.038[5]

[a–c]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]SA = stearic acid; LA = lauric acid; MA = myristic acid.

[2]CLA = conjugated linoleic acid.

[3]ND = not detected.

[4]MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

[5]LA vs. SA, $P = 0.103$.

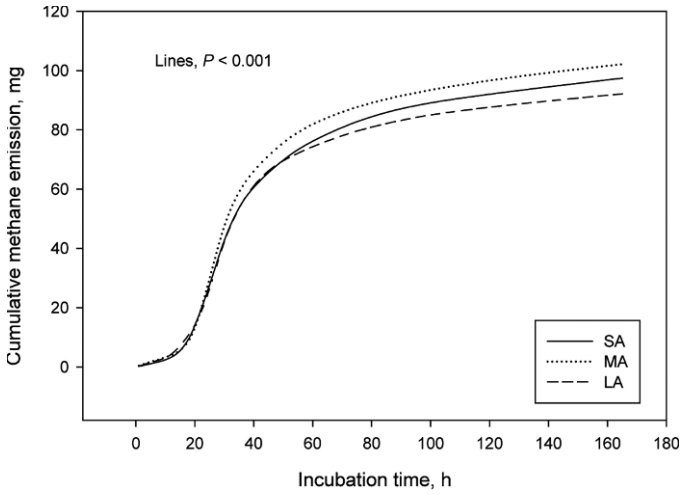

Figure 5. Effect of stearic (SA), lauric (LA), and myristic acids (MA) on cumulative methane emission from dairy manure. End-point (165 h) cumulative methane emission (n = 18; $P = 0.49$): 97, 92, and 102 mg (SA, LA, and MA, respectively). Comparison of regression lines (n = 3,366), $P < 0.001$.

dietary nutrients for milk protein and lactose synthesis than cows on SA. Similar trends for increased milk feed efficiency with LA or coconut oil were observed by Faciola et al. (2008; 1.37 vs. 1.55 kg/kg, control and highest levels of LA) and Hollmann and Beede (2008; 1.58 vs. 1.62 kg/kg for control and 2.5% coconut oil, respectively). Due to the milk fat depression effect of LA, however, when expressed on an FCM or milk NE_L basis, feed efficiency was not different between LA and the other treatments.

Lauric and myristic acids had no effect on ruminal methane production in this experiment. Methane production was reduced with MA (50 g/kg of dietary DM) in the experiment by Machmüller et al. (2003). Such high application levels, however, cannot be considered a practical approach for reducing methane emissions from ruminants. The lack of effect of LA (or MA) on methane production in this experiment is difficult to explain. The intention of the study was to identify which of the 2 FA is responsible for the methane-suppressing effect of coconut oil (Hristov et al., 2009). Similar to our observations, in beef cattle fed about 48% forage diet, coconut oil supplemented at 250 g/d suppressed methane production by about 20%, an effect accompanied by a 63% reduction in protozoal counts (Jordan et al., 2006). A similar antiprotozoal and methane-mitigating effect was observed for the other source of LA and MA in this study, copra meal. The effect of LA and MA on rumen methanogenesis, however, has been inconsistent [the reader is referred to Hristov et al. (2009) for an extended discussion on the topic]. It was expected that LA would exert a methane-inhibitory effect through its strong antiprotozoal activities. This, however, was not the case in Hristov et al. (2009) or in this study. This is the first study in which we evaluated the rumen effects of MA, and its methane-suppressing effect is apparently nonexistent (at the application level studied). It must be pointed out that the application level of coconut oil in Hristov et al. (2009) was considerably higher than for any of the FA in this experiment. Therefore, we conclude that the antimethanogenic effect of coconut oil reported by Hristov et al. (2009) is likely due to (1) greater application level of total MCSFA and (2) a possible additive effect of LA and MA, as neither of these FA exhibits antimethanogenic effects individually. Another puzzling observation in this and the Hristov et al. (2009) reports is that, despite the dramatic decrease in protozoal counts, methanogen-specific DGGE did not reveal any treatment-specific banding patterns. This is in apparent contrast with reports that protozoal-associated methanogens account for up to 90% of the ruminal methanogen population in the rumen (Sharp et al., 1998). Again, a detailed discussion on this discrepancy was provided in Hristov et al. (2009), but it is likely that hydrogen concentration, a measure missing in this experiment, rather than methanogen populations, might be a key factor regulating methane production in the rumen. These data also question the applicability of the DGGE technique for quantitative analyses of methanogenic populations in ruminal contents in vivo.

Ruminal concentration of nitrous oxide, a potent GHG, was very low in this experiment, similar to our previous observations (Hristov et al., 2010), but was reduced by LA, which corresponded to the reduced ammonia concentration with that treatment and may be indicative of ammonium oxidation processes (Yoshida and Alexander, 1970). The global-warming potential of nitrous oxide for the 100-yr time horizon is 298 times that of carbon dioxide, or about 10 times the global warming potential of methane (Forster et al., 2007). Thus, on a carbon dioxide-equivalent basis, nitrous oxide emissions from the rumen in this study would be about 2 to 3% of that of methane.

Milk FA composition reflected the administration of treatment FA with increases in concentration of SA, LA, or MA. The implications of such a significant increase in milk MCSFA to human health have been discussed in detail (Hristov et al., 2009). Stearic acid was chosen as control in this experiment because of its minimal effects on ruminal fermentation (Noble et al., 1969; Hristov et al., 2004a). Stearic acid, however, included at 5 or 10% in the concentrate mixture had profound effects on milk FA composition; C18:0 and 18:1 were increased and concentrations of shorter chain FA (from C6:0 to C10:0, but also C14:0, 14:0, 14:1, and 16:0), as well as C18:2, were decreased (Noble et al., 1969). The

MA treatment increased 14:1 in addition to 14:0, and this presumably resulted from mammary stearoyl-CoA desaturase activity. An analogous increase in 18:1, *cis*-9 was not observed with SA despite increases in milk fat 18:0. Profound changes in the concentration and profile of milk fat *trans*-18:1 and CLA isomers were observed with LA. These changes tend to be associated with milk fat depression and this treatment did reduce milk lipogenesis, reducing milk fat concentration by about 25% compared with SA. As pointed out by Shingfield and Griinari (2007), CLA isomers such as *trans*-10, *cis*-12 had been implicated in milk fat depression. In this study, however, *trans*-10, *cis*-12 concentration was negligible (although increased significantly by LA). Concentration of *cis*-9, *trans*-11, on the other hand, was almost doubled by LA, reaching 1.1% of total milk fat, and may indicate production of other CLA isomers not measured or detected that could result in milk fat depression (Shingfield and Griinari, 2007). No statistically significant changes in CLA isomers were observed for MA, which produced only a numerical decrease in milk fat. Other changes in milk FA composition, including reductions in shorter-chain FA also are characteristic of this situation. Alterations in *trans*-18:1 and CLA isomers indicate that LA influenced ruminal biohydrogenation and, based on reductions in 18:0, resulted in diminished reduction of biohydrogenation intermediates to the terminal product of biohydrogenation, 18:0. The dramatic increase in *trans*-18:1 by LA compared with both SA and MA is in agreement with diet-induced milk fat depression (Shingfield and Griinari, 2007). The discrepancy between the influence of LA on ruminal microbes in comparison to MA is unresolved.

CONCLUSIONS

Lauric acid had a dramatic effect on ruminal fermentation in this study. Protozoal counts were reduced by 96% and the effects on VFA concentrations and estimated microbial protein production were indicative of general suppression of microbial activities. No such effects were observed for myristic acid. Both lauric and myristic acids had no effect on ruminal methane production, which, for lauric acid, is in contrast to the observed significant reduction of protozoal counts. This and the lack of effect of treatment (specifically lauric acid) on methanogen-specific DGGE banding patterns may be indicative of other factors, such as hydrogen accumulation, important in regulating methane production in the rumen. Lauric acid depressed DMI and consequently milk yield, and also caused milk fat depression. All treatments increased milk concentration of the respective fatty acid. Concentrations of saturated fatty acids in milk were reduced and that of >C16 and MUFA were increased by lauric acid. Lauric acid increased concentrations of *trans*-18:1 FA and CLA isomers, which was the likely causative factor for the observed milk fat depression with this treatment.

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